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Effect of Nonablative Laser Energy on the Joint Capsule: An In Vivo Rabbit Study Using a Holmium:YAG Laser

Kei Hayashi, DVM, MS,[1] Janet A. Nieckarz, BS,[1] George Thabit III, MD,[2] John J. Bogdanske, BA,[1] A.J. Cooley, DVM,[1] and Mark D. Markel, DVM, PhD[1]*

[1]Comparative Orthopaedic Research Laboratory, School of Veterinary Medicine, University of Wisconsin, Madison 53706
[2]Sports, Orthopedic and Rehabilitation Medicine Associates, Menlo Park, California 94025

Background and Objective: **The nonablative application of holmium:yttrium-aluminum-garnet (Ho:YAG) laser energy to the joint capsule of patients with glenohumeral instability has been found to shrink capsular tissue and to help stabilize the joint. The purpose of this study was to evaluate the effect of nonablative laser energy on the short-term histological properties of joint capsular tissue in an in vivo rabbit model.**
Study Design/Materials and Methods: **Eighteen mature New Zealand white rabbits were used in this study. One randomly selected stifle was treated with laser energy, and the contralateral stifle was sham-operated. Animals were euthanized immediately after surgery (day 0), at 7 days postsurgery and 30 days postsurgery. Specimens were processed for histology and transmission electron microscopy.**
Results: **Laser-treated samples at day 0 showed diffuse hyalinization of collagen with nuclear karyorrhexis of fibroblasts. Laser-treated tissue at 7 days postsurgery revealed fibroblast proliferation around and into acellular hyalinized regions of collagen. At 30 days postlaser treatment, areas of fused collagen were greatly reduced as large reactive fibroblasts migrated and secreted matrix.**
Conclusion: **This study illustrates the short-term in vivo tissue response to nonablative laser treatment, where acellular hyalinized regions of collagen are infiltrated by fibroblasts that have used the treated collagen as the framework for migration and secretion of new collagen matrix in order for tissue repair to proceed. Lasers Surg. Med. 20:164–171, 1997.**

Key words: collagen; fibroblast; histology; tissue response; transmission electron microscopy

INTRODUCTION

A recent pilot study has demonstrated that the nonablative application of the holmium:yttrium-aluminum-garnet (Ho:YAG) laser energy to the joint capsule of patients with glenohumeral instability shrank the joint capsule, stabilizing the shoulder in the majority of the patients treated [1]. Glenohumeral instability secondary to ligamentous laxity, capsular redundancy, and excessive

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*Correspondence to: Mark D. Markel, DVM, Comparative Orthopaedic Research Laboratory, Department of Medical Sciences, School of Veterinary Medicine, University of Wisconsin, 2015 Linden Drive West, Madison, WI 53706.

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joint volume is a frequent occurrence [2–5] that current closed, open, and arthroscopic treatments do not address satisfactorily in certain subgroups [4, 6–10]. In a multi-institutional clinical trial [1], nonablative application of the Ho:YAG laser, which has been approved for arthroscopic surgery, was applied to patients with glenohumeral instability without capsulolabral detachment or full-thickness rotator cuff tears. Laser energy was applied tangentially with the unit set at 10 watts (1.0 J, 10 pulses/sec) to shrink the capsuloligamentous tissues of the glenohumeral joint without ablation. For all patients, regardless of arm dominance, age, sex, or direction of instability, postsurgical subjective scores were significantly higher than presurgical scores. Although this study suffered from lack of a comparable nonoperated control population or an operated open surgical repair group, these results indicate that at this short-term follow-up (mean 6 months), patients in this subgroup improved dramatically after nonablative reduction of redundant glenohumeral joint capsule using the Ho:YAG laser.

We previously reported that Ho:YAG laser energy at nonablative levels can significantly alter joint capsular length and its mechanical and histological properties in an in vitro rabbit study [11]. Laser treatment significantly shortened the tissue by 9% (5 watts: 0.5 J/10 Pulses per sec), 26% (10 watts: 1.0 J/10 pulses per sec), and 38% (15 watts: 1.5 J/10 pulses per sec), respectively. Histological analysis of the tissue revealed significant thermal alteration of collagen and fibroblasts in the laser treatment groups, with each subsequently higher laser energy causing significantly greater morphologic change over a larger area. [12]

These results suggested that the predominant effect of nonablative laser energy on joint capsular tissue is thermally mediated. Thermal damage can cause denaturation of collagen and necrotic changes of fibroblasts. Although a pilot clinical study suggested the effectiveness of laser treatment in patients with glenohumeral instability, to date no studies have been performed examining the histological and ultrastructural properties of joint capsular tissue following the application of laser energy. The purpose of this study was to evaluate the effect of tissue response on the laser-induced alterations of joint capsular tissue. Specifically, we evaluated the effect of nonablative Ho:YAG laser energy on the short-term histological and ultrastructural properties of joint capsular tissue in an in vivo rabbit model.

MATERIALS AND METHODS

Eighteen mature New Zealand white rabbits, ranging in weight from 4.3–6.5 kg (4.7 ± 0.52; mean ± SD), were used in this study. This study was approved by the Institutional Animal Use and Care Committee. Rabbits were randomly assigned to one of three groups (0, 7, and 30 days postsurgery). The animals were anesthetized with halothane and oxygen, and both stifles of each rabbit were aseptically prepared for surgery. The femoropatellar joint was exposed via a patellar tenotomy. One randomly selected stifle was treated with laser energy using a Ho:YAG laser (VersaPulse, Coherent, Palo Alto, CA) and a 1.7 mm hand piece (InfraTome, Coherent, Palo Alto, CA), and the contralateral stifle was sham-operated. A custom-designed jig that allowed delivery of the laser energy in a lactated Ringer's solution bath was used. Laser energy (5 watts: 0.5J per pulse / 10 pulses per second) was applied to the medial and lateral compartments of the femoropatellar joint capsule in a defocused manner. The laser handpiece was held ~1.5 mm from the synovial surface by a custom-designed jig and moved over the tissue in a paintbrushlike motion. Following the procedure, the joint capsule, subcutaneous tissue, and skin were closed routinely.

Animals were euthanized at three time intervals: immediately after surgery (day 0), 7 days postsurgery, and 30 days postsurgery. The medial and lateral portions of the femoropatellar joint capsule were harvested immediately after euthanasia. Specimens were processed for histology and transmission electron microscopy. Tissue samples for histology were fixed in neutral-buffered 10% formalin, embedded, sectioned on the plane perpendicular to the synovial surface of the specimen, and processed for histological staining with hematoxylin-eosin. Tissue samples for transmission electron microscopy were fixed in modified Karnovsky's solution (2% paraformaldehyde and 1.25% glutaraldehyde in 0.1 M sodium phosphate buffer, pH 7.0), stored in 0.1 M sodium phosphate buffer for 8 hr at 4°C, postfixed for 2 hr in 1% osmium tetroxide, and stained with 1% uranyl acetate. After sequential dehydration in ethanol and infiltration in epon-araldite and propylene oxide, specimens were embedded in 100% epon-araldite and polymerized at 60°C. Thick (1 μm) and ultrathin (70 nm) sections were cut for light and electron microscopy, respectively. The ultrathin sections were placed on grids, stained with lead citrate and viewed using a transmission electron microscope.

RESULTS

Histology

Control tissues obtained from animals euthanized immediately after sham operations showed no significant histological lesions in the joint capsule tissue (Fig. 1a). Laser-treated samples at day 0 showed significant histological alterations with diffuse hyalinization and fusion of collagen fibers along with nuclear karyorrhexis and nuclear streaming of fibroblasts throughout the treated regions (Fig. 1b). Control tissues at 7 days postsham operation showed granulation tissue, mild fibrosis, and mixed inflammatory infiltration including lymphocytes, plasma cells, and heterophils (Fig. 1c). Laser-treated tissue at 7 days postsurgery revealed a similar inflammatory response to control tissues along with fibroblast proliferation around and into multifocal acellular hyalinized collagen regions (Fig. 1d). Normal fibrous collagen was present in the regions adjacent to the acellular treated region with increased numbers of large rounded fibroblasts (Fig. 1d). Control tissue at 30 days postsham operation showed mature granulation tissue and regular dense fibrous connective tissue in the normal collagenous joint capsule tissue (Fig. 1e). Laser-treated tissues at 30 days postsurgery showed fibrosis with cellular and disorganized connective tissue. Fused collagen regions were greatly reduced by 30 days postlaser treatment as large fibroblasts migrated to the site and secreted new collagen matrix to replace the hyalinized tissue (Fig. 1f). For both laser-treated and sham-operated groups at 7 and 30 days postsurgery, there were variations in the degree of inflammatory reaction within groups, although the responses to the laser treatments were similar within laser-treated groups.

Electron Microscopy

Transmission electron microscopy revealed no significant ultrastructural alterations in collagen or fibroblast architecture in control tissues obtained immediately postsham operations (Figs. 2, 3). The typical appearance of cross-sectional regions at day 0 revealed collagen fibrils of a variety of sizes with distinct margins (Fig. 2a). Longitudinal sections of control tissue collagen fibrils showed normal periodical cross-striations and normal quiescent spindle shaped fibroblasts with large condensed nuclei and sparse cytoplasm with no ultrastructural evidence of active secretion (Fig. 2a). Tissue samples obtained immediately after laser treatment revealed significant alterations in collagenous and fibroblast ultrastructure (Figs. 2b, 3a). Cross-sectional regions showed increases in fibril diameter with a loss of distinct fibril margins and longitudinal sections revealed increased fibril diameter with the loss of cross striations (Fig. 2b). Fibroblasts in laser treated areas were pyknotic with evidence of nuclear karyorrhexis and nuclear streaming resulting from disruption of the nuclear and cellular membrane (Figs. 2b, 3a).

Control tissues at 7 days postsham operation showed no ultrastructural alterations in collagen fibrils; however, some active fibroblasts with increased rough endoplasmic reticulum and secretory vesicles were noted. Tissue samples at 7 days postlaser treatment indicated significant ultrastructural alterations of collagen and fibroblasts relating to the tissue response and repair process (Figs. 2c, 3b,c). Areas directly treated with laser energy showed loss of cellularity and evidence of cellular degeneration (Figs. 2c, 3b). In this acellular region, striated collagen fibrils and agglomerates of polymerized microfibrils were observed (Figs. 2c, 3b). Metabolically active fibroblasts were noted to be most predominate adjacent to these treated acellular regions (Fig. 3c). Electron microscopy in this area showed increased active fibroblasts and surrounding small collagen fibrils. Fibroblasts revealed an increase in nuclear and cytoplasmic area with elaborate rough endoplasmic reticulum, polyribosomes, mitochondria, and secretory vesicles.

Control samples at 30 days postsham operations revealed no significant collagen or fibroblast ultrastructural changes. Samples obtained at 30 days postlaser treatment indicated that laser-treated regions had increased cellularity with enlarged fibroblasts with extensive cytoplasm that showed an increased number of secretory vesicles along the plasma membrane and an increased arrangement of rough endoplasmic reticulum, golgi apparatus, and mitochondria (Figs. 2d, 3d). Electron microscopy of cross-sectional regions at the treatment interface showed very small collagen fibrils interspersed with larger diameter fibrils and increased active fibroblast cellularity (Figs. 2d, 3d). Longitudinal sections of collagen revealed both large collagen fibrils and finer fibrils with striations and large active fibroblasts with increased cytoplasmic organelles (Fig. 2d).

DISCUSSION

This study illustrates the histological and ultrastructural alterations of the in vivo tissue re-

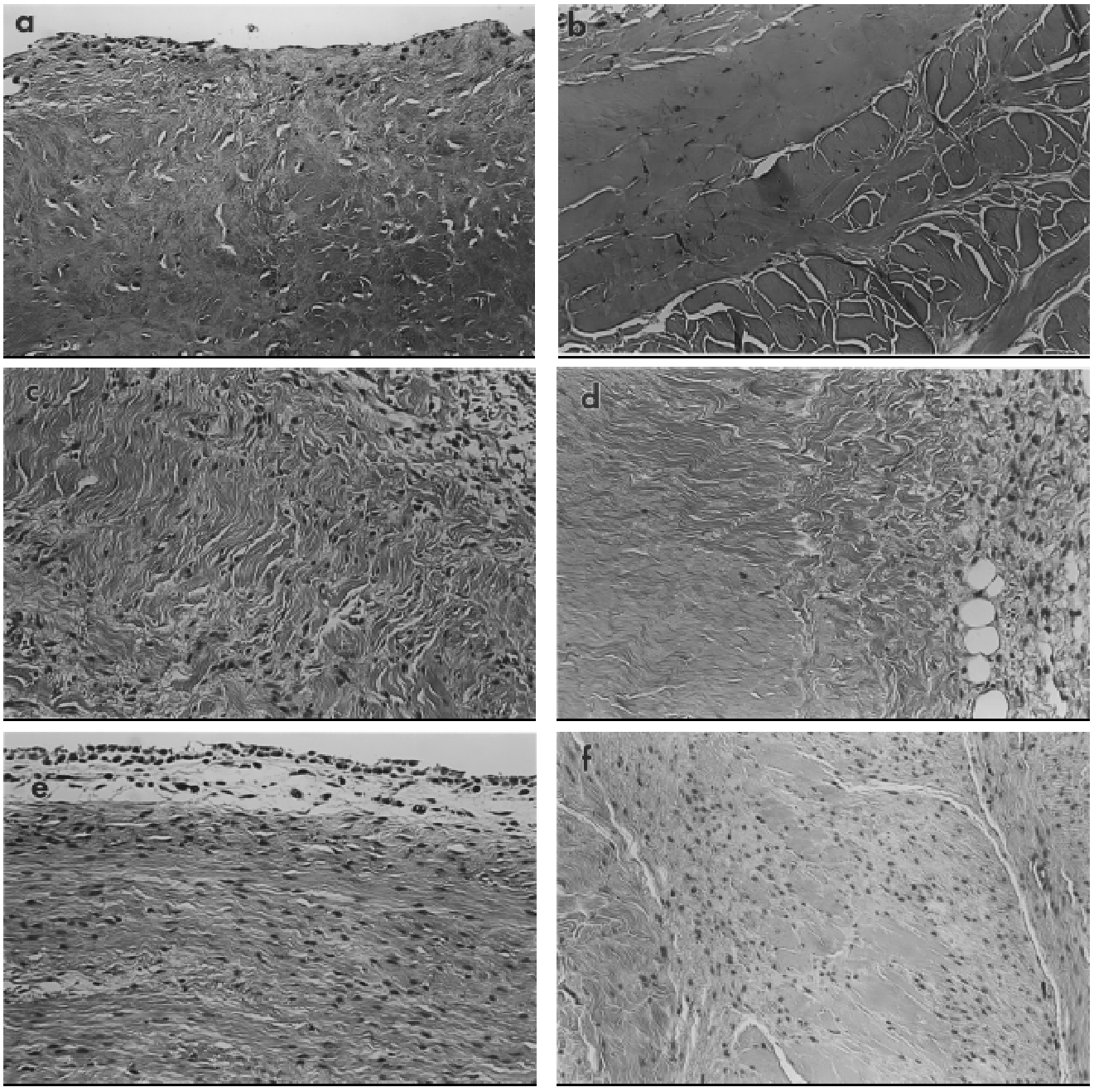

Fig. 1. Light micrograph of (**a**) control day 0 capsular tissue demonstrating normal collagen and fibroblasts, (**b**) laser-treated day 0 capsular tissue demonstrating hyalinization of collagen and karyorrhexis of fibroblasts, (**c**) control day 7 tissue demonstrating a mild inflammatory response, (**d**) laser-treated day 7 tissue demonstrating acellular treated region and adjacent fibrous collagen and active fibroblasts, (**e**) control day 30 tissue demonstrating regular fibrous connective tissue, and (**f**) laser-treated day 30 tissue demonstrating greatly reduced hyalinized acellular regions with surrounding fibroblasts and fibrosis (hematoxylin-eosin stain, original ×50).

sponse and collagen repair process following nonablative laser treatment of joint capsular tissue. The nonablative applications of laser energy have been evaluated primarily in tissue welding and thermokeratoplasty [13–16]. Rabau et al. [17] evaluated the healing process of laser-welded intestinal anastomoses in a rat model as compared with sutured anastomoses. The investigators reported that despite significantly lower DNA and collagen concentrations at the 4th postoperative day, collagen concentrations on the 7th and 10th postoperative days were significantly higher in

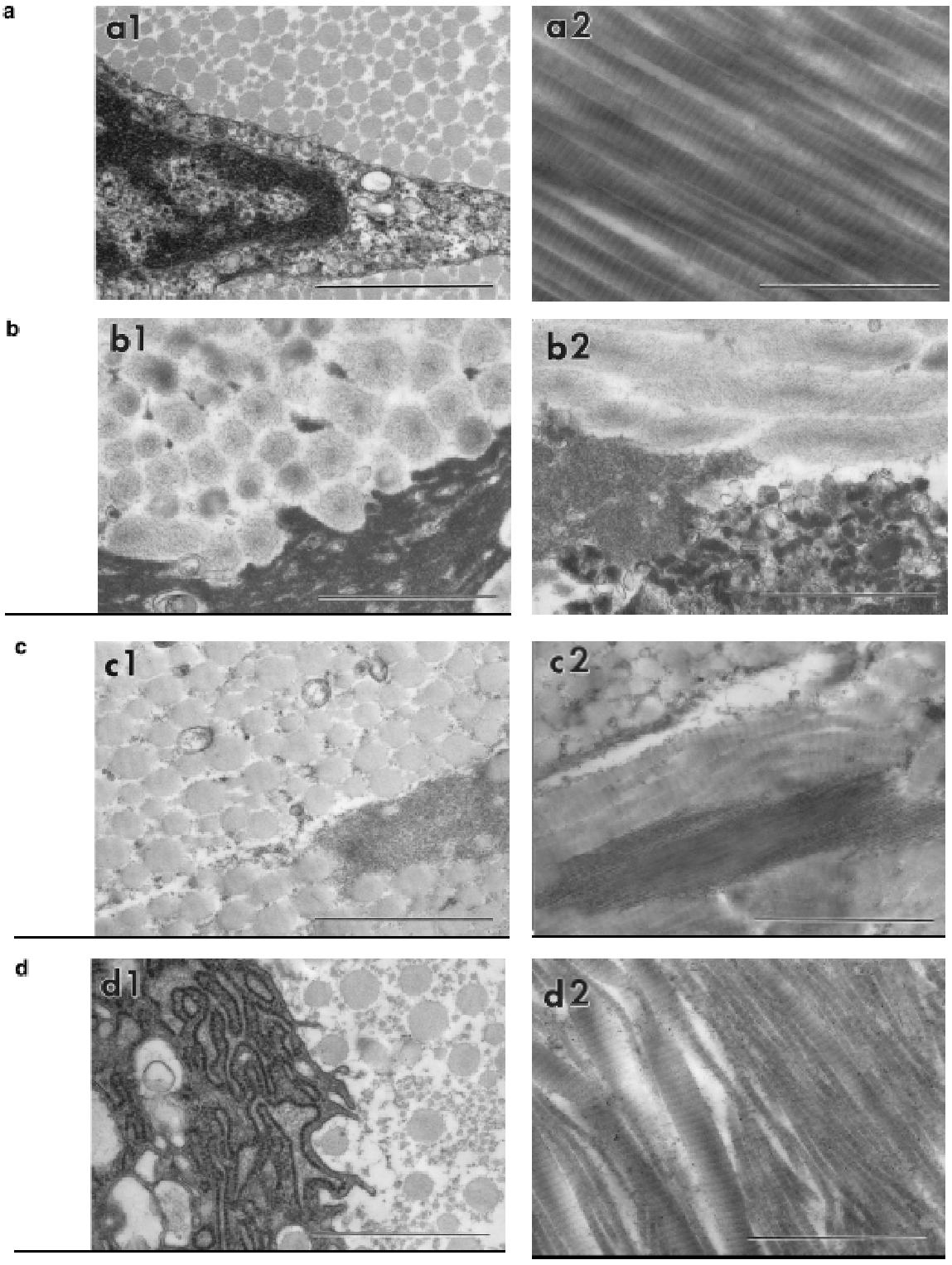

Fig. 2. Transmission electron microscopy of (**a**) control day 0 joint capsular tissue demonstrating a normal fibroblast and a variety of sizes of distinct collagen fibrils (a1: cross section) with characteristic periodical cross striations (a2: longitudinal section), (**b**) laser-treated day 0 joint capsular tissue demonstrating pyknotic fibroblasts with loss of membrane integrity and collagen fibrils with increased diameter and loss of distinct edges (b1) and loss of longitudinal cross-striations (b2), (**c**) laser-treated day 7 joint capsular tissue in the treated area demonstrating loss of cellularity with evidence of cellular degradation (c1) and striated collagen fibrils with microfibrillar structures (c2), and (**d**) laser-treated day 30 joint capsular tissue at the treatment interface demonstrating small collagen fibrils interspersed with large fibrils around an active fibroblast (d1, d2) (×24,000, bar = 1 μm).

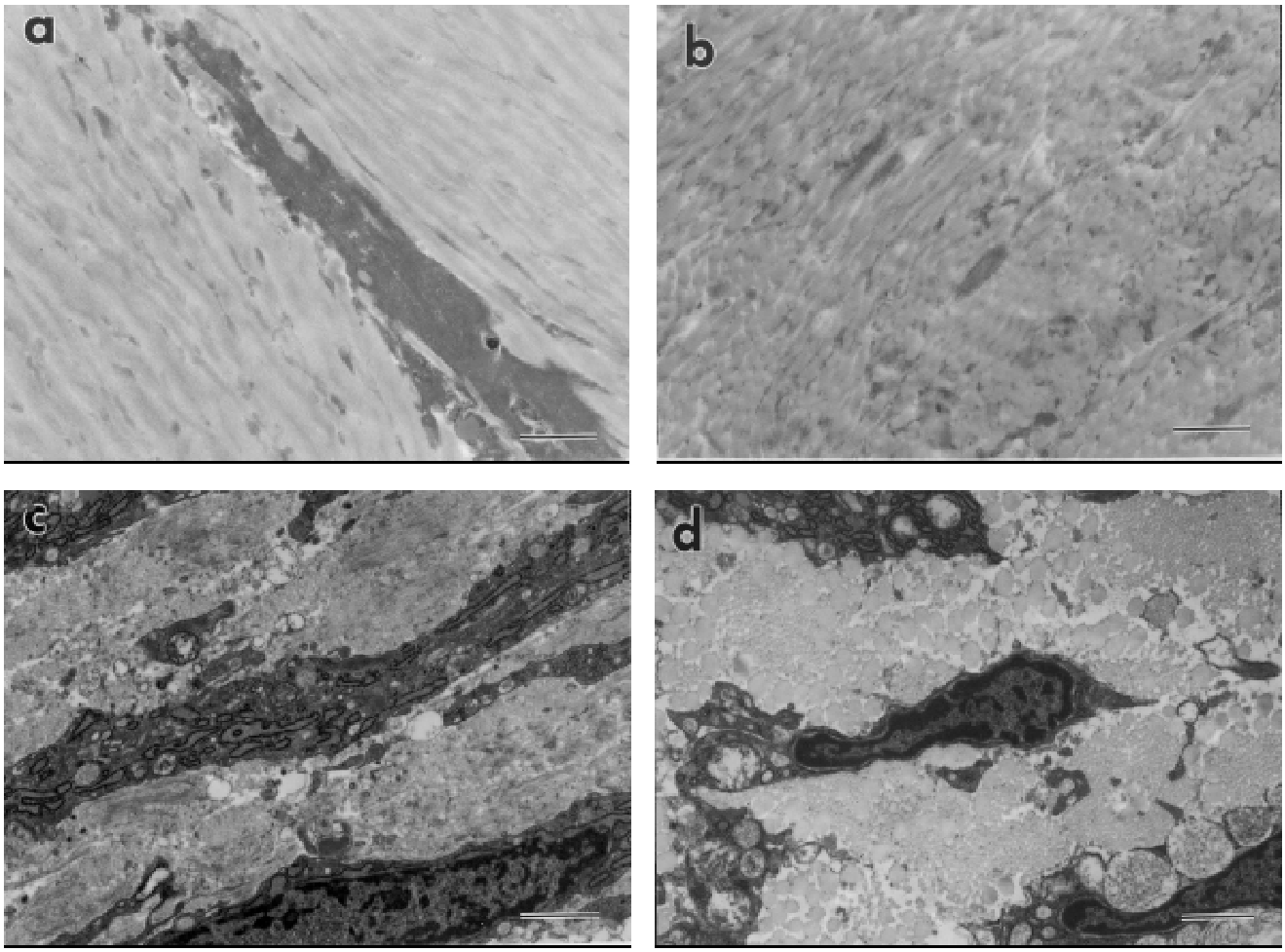

Fig. 3. Transmission electron microscopy of (**a**) laser-treated day 0 joint capsular tissue demonstrating a pyknotic fibroblast and collagen fibrils with loss of striations, (**b**) laser-treated day 7 joint capsular tissue in the treated area demonstrating striated collagen fibrils and loss of cellularity, (**c**) laser-treated day 7 joint capsular tissue in the area adjacent to the treated area demonstrating increased numbers of active fibroblasts, and (**d**) laser-treated day 30 joint capsular tissue demonstrating large and small collagen fibrils around active fibroblasts (×6,900, bar = 1 μm).

the laser treated group than in a sutured group. They attributed this result to less inflammatory reaction following more rapid fibroblast proliferation in the laser treated group. To date, in vivo studies have not been performed examining the effect of tissue healing on the histological and ultrastructural properties of joint capsular tissue following the application of nonablative laser energy.

Histology of laser-treated samples at day 0 revealed significant fusion and hyalinization of collagen caused by thermal damage of the laser treatment. At 7 days postlaser treatment, acellular hyalinized regions of collagen were infiltrated with large rounded fibroblasts. Fibrosis with cellular randomly arranged connective tissue continued to replace the treated tissue at 30 days postlaser application, which significantly reduced the area of hyalinized collagen as seen via light microscopy. Both control and laser-treated samples showed some level of inflammatory infiltration, fibrosis, and granulation tissue invasion postsurgically, indicating that the sham operation probably resulted in a mild inflammatory response. Transmission electron microscopy revealed collagen and fibroblast alterations that further support the histological tissue response. As noted in previous studies [18], the most significant change in collagen observed at day 0 postlaser treatment was the disruption of regular fibril organization, which was demonstrated as an increase in fibril diameter and the loss of the fibril's distinct edge on cross-section and the loss of periodical cross-striations on longitudinal section. Although histologically

the collagen bundles appeared fused with light microscopy, electron microscopy revealed that individual circular collagen fibrils were still evident. We hypothesized that these changes in collagen are mainly due to denaturation of collagen caused by the thermal effect of laser energy. Heat-induced shrinkage associated with denaturation of collagen is a well-described phenomenon [13–16, 19–23]. At shrinkage temperatures, thermal unwinding of the triple helices outweighs the constraints of natural crosslinks, causing the fibrils to denature and shrink [19]. Fibroblastic morphology was altered in the laser-treated sites with both pyknotic changes and loss of cytoplasmic and nuclear membrane integrity evident. These changes were most likely caused by the thermal and/or mechanical effects of laser energy.

The synthesis, accumulation, and degradation of collagen are dynamic processes that occur intracellularly and extracellularly during morphogenesis, growth, inflammation, and repair [24–27]. In this study, evidence of active tissue healing was observed at 7 days and 30 days postlaser treatment. At the interface of the treated regions and normal tissue, increased numbers of actively secreting fibroblasts were present, which was established by an increase of cytoplasmic organelles, including rough endoplasmic reticulum, mitochondria, golgi, and secretory vesicles. Fine collagen fibrils adjacent to laser-altered fibrils may provide evidence of newly secreted collagen matrix and tissue repair. It appears that reactive fibroblasts migrate into the treated regions using the larger denatured collagen fibrils as a scaffold in order to initiate collagen repair. Over time in laser-treated sites, pyknotic nuclei fragmented and degraded to form acellular regions. No macrophages or phagocytic cells were evident within the treated region; however, active fibroblasts were significantly increased adjacent to and infiltrating these areas.

In this study, laser energy (5 watts: 0.5J per pulse / 10 pulses per sec) was applied to the medial and lateral compartments of the femoropatellar joint capsule in a defocused manner in a paintbrushlike motion using a custom-designed jig that allowed delivery of the laser energy in a lactated Ringer's solution bath ~1.5 mm away from the synovial surface. The distance from the tip of the handpiece to the tissue was relatively well controlled; however, in addition to the distance, other factors such as angle of the beam, spot size, and intervening solution temperature and thermal conductivity would also play important roles. Clinically, it would be much more difficult to deliver laser energy at nonablative levels without inadvertently overheating some regions and underheating other regions. Although the histological alterations of the tissue by laser application were similar within laser treatment groups, further improvements in the method of energy delivery may be necessary.

This study illustrates the short-term, in vivo tissue response to laser-treated joint capsular tissue in the rabbit model. Histological and ultrastructural examination revealed alterations in both collagen and fibroblast morphology in laser-treated regions as demonstrated by hyalinized collagen with pyknotic cells and nuclear streaming, and indistinct enlarged collagen fibrils with loss of cross striations. At 7 days postlaser treatment, large acellular region of hyalinized collagen with surrounding large fibroblasts was the predominant feature at the treated site. Histology revealed a significant reduction in the area of hyalinized collagen at 30 days postlaser treatment with increased fibroblast proliferation and fibrosis. Electron microscopy supported this histological finding in which metabolically active fibroblasts with increased cytoplasmic area, including rough endoplasmic reticulum, golgi apparatus, mitochondria and secretory vesicles, were evident. Small collagen fibrils were also significantly increased and interspersed with larger collagen fibrils in previously treated areas. This study demonstrated that active healing is ongoing with a residual population of fibroblasts at the end of this experimental period. This finding supports the concept that new collagen is actively synthesized around treated collagen fibrils, although this study did not clarify whether the denatured collagen is entirely degraded or some areas of altered collagen may return to their original fibril organization and function. Further long-term in vivo studies are needed to evaluate the condition of collagen and fibroblasts and the synthetic activity of fibroblasts on collagen after thermal treatment by nonablative laser energy.

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